DNA VACCINE CONTAINING TUMOR-ASSOCIATED GENE AND CYTOKINE GENE AND METHOD OF PREPARATION THE SAME

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a DNA vaccine and its preparation, which employs DNA recombination technique to co-incorporate at least one fragment of tumor-associated gene and at least one fragment of cytokine gene into a vector, thereby producing a DNA vaccine containing at least one fragment of tumor-associated gene and at least one fragment of cytokine gene simultaneously.

Description of Related Art

The immunotherapy ~~of~~ for cancer is drawing more and more attention in recent years. In particular, ~~along with~~ due to the development of molecular biology and the ~~advancement~~ progress of biotechnology, there has been a ~~the making of cancer vaccines has seen~~ significant breakthrough in DNA vaccine development. ~~Currently the~~ Current available ~~types of~~ cancer ~~vaccine available~~ vaccines include at least DNA vaccine, dendritic cell vaccine and gene-modified tumor vaccine etc. Unlike a typical vaccine ~~that is~~ used ~~to~~ for ~~prevent a~~ disease prevention, cancer vaccine aims at ~~treating~~ cancer treatment. More specifically, the purpose of cancer vaccine is to boost ~~the body~~ self-immunity ~~immunity to~~ for tumor cells and enable the immune system to recognize ~~and~~ as well as kill tumor cells. ~~Wherein~~ DNA vaccine transforms ~~introduces gene~~ genes encoding specific tumor-associated ~~antigen~~ antigens (e.g. oncogene such as neu, met or ras) into ~~the~~ host ~~cell~~ cells where said tumor-associated ~~antigen~~ antigens ~~is~~ are expressed through ~~the mechanism of~~ transcription and translation to ~~elicit~~ induce the immune response of the host against said tumor-associated ~~antigen~~ antigens and to

~~achieve the effect of inhibiting~~ inhibit or suppress ~~retarding~~ the growth of tumor cells.

~~In the example of~~Take oncogene neu (also ~~called~~ known as Her-2 or c-erbB-2) as an example, previous studies ~~found~~ indicated that neu gene was ~~over-expressed~~ over-expressed in the tumor tissues of some patients with lung cancer, breast cancer, ovarian cancer or bladder cancer. ~~The oncogene~~ Oncogone neu encodes a transmembrane glycoprotein, which is a growth factor receptor ~~that can receive message~~ receiving signals ~~of~~ to expedite ~~accelerating~~ cell growth and division. Since ~~Given the positive correlation between~~ the overexpression of neu gene and propagation of tumor ~~cell~~ cells are positively related, ~~it~~ neu gene may be ~~treated~~ considered as a tumor-associated antigen. In addition, the overexpression of neu gene is also related to drug resistance in ~~chemotherapy~~ medication;~~. Patients~~ patients with such ~~condition~~ symptom usually have poor prognosis.

~~Exactly because~~ Because of its overexpression in certain types of cancer, neu gene ~~may~~ can be used to design cancer vaccines ~~that target~~ targeting the gene ~~it~~ itself, for instance, a DNA vaccine ~~that carries~~ carrying neu gene. The ~~combined use~~ combination of neu DNA vaccine and cytokine-specific tumor vaccine, such as Interleukin-2 (IL-2), Interleukin-4 (IL-4), and GM-CSF (granulocyte macrophage colony-stimulating factor) has ~~been~~ shown its capability to inhibit the growth of tumor cells in mice. ~~But~~ However, the preparation of such tumor vaccine requires prolonged in vitro culture and an extra ~~screening of~~ tumor cells screening process ~~in vitro~~. ~~In the~~ While culturing ~~process~~, mutation is prone to occur ~~that~~ and results in the loss of surface antigen; while ~~in the~~ screening ~~process~~, the heterogeneity of the tumor cells might decrease ~~be reduced that~~ and reduce ~~narrows~~ the protection ~~range~~ of tumor vaccine. ~~Plus~~ Moreover, ~~the fact that~~ it is costly to prepare this ~~kind~~ type of vaccine, and its clinical application is still not popular so far ~~has been limited~~.

SUMMARY OF THE INVENTION

In order to ~~addressing~~ address the drawbacks of prior arts, the present invention ~~aims to provide~~ provides an easily prepared and relatively low-cost DNA vaccine and ~~its~~ the method of ~~preparation~~ preparing the same ~~process~~. ~~The foregoing~~ Aforesaid DNA vaccine is prepared by ~~incorporating~~ implanting at least one fragment of tumor-associated gene and at least one fragment of cytokine gene into a vector that contains ~~a~~ one or more suitable ~~promoter~~ promoters and/or ~~or~~ one or more translation ~~regulatory~~ regulating sequence~~; at least one fragment of tumor-associated gene; and one fragment of cytokine gene~~. The resulting DNA vaccine contains ~~both~~ one or more tumor-associated ~~gene~~ genes and one or more cytokine ~~gene~~ genes.

The expression of tumor-associated ~~gene~~ genes and cytokine ~~gene~~ genes ~~incorporated~~ implanted into the aforesaid vector may be ~~controlled~~ regulated by one or more mammalian ~~expression~~ promoters and/or ~~or~~ ~~regulated by~~ the IRES (internal ribosome entry site).

Another purpose ~~objective~~ of the present invention is to provide a method ~~of~~ to ~~preparing~~ construct a DNA vaccine containing at least one fragment of tumor-associated gene and at least one fragment of cytokine gene, comprising ~~at least the following steps~~: designing ~~a primer sequence containing proper~~ primers with specific restriction ~~site~~ sites; ~~using~~ amplifying said tumor-associated gene fragments and cytokine gene fragments by polymerase chain reaction (PCR) ~~to amplify~~ and ~~isolate~~ isolating the two fragments ~~aforesaid tumor-associated gene and cytokine gene~~ respectively; ~~using~~ ligating the tumor-associated genes and cytokine genes with a vector containing one or more promoters and/or one or more translation regulating sequences by ligase ~~ligase to co-incorporate respectively the tumor-associated gene and cytokine gene into a vector having a suitable promoter or translation regulatory sequence~~.

The aforesaid tumor-associated ~~gene~~ genes and cytokine ~~gene~~ genes on the vector may be arranged in such an order that the tumor-associated ~~gene~~ genes ~~is~~ are located in front of or in back of ~~behind~~ the cytokine ~~gene~~ genes.

The aforesaid ~~co-incorporation~~ construction method comprises ~~can be achieved~~ at least ~~by way of~~: combining the tumor-associated ~~gene~~ genes and the cytokine ~~gene into~~ genes as a fusion gene ~~to be~~ controlled by ~~the same~~ one promoter; or having two independent genes ~~that are~~ controlled respectively by two separate promoters; or having two independent genes ~~that are respectively controlled~~ regulated by a promoter and ~~regulated by~~ an IRES ~~segment~~ respectively.

The aforesaid DNA vaccine may be ~~carried~~ transformed at least by retroviral vector, adenoviral vector, adeno-associated viral vector, or liposome, or administered directly in the form of DNA.

The aforesaid DNA vaccine may be administered at least by ~~way of~~ subcutaneous injection, intramuscular injection, oral administration, spraying or gene gun injection.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 ~~is a diagram of~~ shows the primer ~~sequence~~ sequences designed ~~in~~ for Embodiment 1 ~~according to~~ in the present invention.

FIG. 2 ~~is a diagram~~ shows ~~of~~ the N neu-IL-2 fusion DNA vaccine in Embodiment 1 ~~according to~~ in the present invention.

FIG. 3 is the ~~experimental~~ flow chart of ~~in~~ Embodiments 2, 3 and 4 in ~~accordance with~~ the present invention.

FIG. 4 ~~is a graph showing~~ shows the tumor-suppressing effect of N neu-IL-2 fusion DNA vaccine ~~as~~ depicted in Embodiment 2 ~~according to~~ in the present invention.

FIG. 5 ~~depicts~~ shows the survival ~~rate~~ rates of mice ~~that received~~ injected ~~respectively the treatment of~~ with ~~normal~~ saline (~~line~~ 5 a), pRc/CMV vector only (~~line~~ 5 b), separate N neu DNA vaccine and IL-2 DNA vaccine ~~given separately~~ (~~line~~ 5 c), N neu DNA vaccine (~~line~~ 5 d) or N neu-IL-2 fusion DNA vaccine (~~line~~ 5 e) ~~as performed~~ in Embodiments 3 and 4 ~~according to~~ in the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a DNA vaccine and its preparation, which employs DNA recombination technique to ~~co-incorporate~~ implant at least a fragment of tumor-associated gene and at least a fragment of cytokine gene into a vector. The administration of an effective dose of such DNA vaccine in ~~a mammal~~ mammalian may enhance the immune response of the ~~host~~ hosts through the co-expression of tumor-associated ~~antigen~~ genes and cytokine genes, and thereby achieve the therapeutic effect of inhibiting or ~~retarding~~ suppressing tumor growth.

The main ~~objective~~ purpose of the present invention is to provide a DNA vaccine, which is prepared by ~~incorporating~~ implanting at least one fragment of tumor-associated gene and at least one fragment of cytokine gene into a vector containing ~~a~~ one or more suitable ~~promoter~~ promoters and/or ~~or~~ one or more translation regulatory ~~sequence at least one fragment of tumor-associated gene and one fragment of cytokine gene~~ sequences. The ~~resulting~~ resulted DNA vaccine contains both tumor-associated ~~gene~~ genes and cytokine ~~gene~~ genes.

The expression of tumor-associated ~~gene~~ genes and cytokine ~~gene~~ genes ~~incorporated~~ implanted into the aforesaid vector may ~~at least be~~ controlled at least by one or more mammalian ~~expression~~ promoters, such as CMV promoter, PSV promoter or LTR promoter, or regulated by IRES. ~~Said~~ Aforesaid tumor-associated ~~gene~~ genes may be full length or truncated ~~a oncogene~~ oncogenes, such as neu, met or ras, ~~in one complete or truncated segment~~, for example, a fragment of N neu gene encoding the extracellular domain of neu protein. ~~Said~~ Aforesaid cytokine ~~gene~~ genes include ~~includes~~ at least ~~the~~ IL-2, IL-4 or GM-CSF gene.

Another ~~objective~~ purpose of the present invention is to provide a method ~~of preparing~~ to construct a DNA vaccine containing at least one fragment of tumor-associated gene and at least one fragment of cytokine gene, comprising ~~at least the following steps~~: designing ~~primer~~ primers ~~sequence~~ containing ~~proper~~ specific restriction ~~site~~ sites; amplifying said tumor-associated gene fragments and cytokine gene fragments by polymerase chain reaction (PCR) and isolating the two fragments ~~using polymerase chain reaction (PCR) to amplify and isolate tumor-associated gene and cytokine gene~~ respectively; ~~using ligase to co-incorporate respectively~~ ligating the tumor-associated ~~gene~~ genes and cytokine ~~gene~~ genes ~~into~~ with a vector ~~having~~ containing ~~a~~ one or more ~~suitable promoter~~ promoters and/or ~~or~~ one or more translation ~~regulatory~~ regulating sequence by ligase.

~~The said~~ Aforesaid tumor-associated ~~gene~~ genes may be located in front of or in back ~~behind the~~ of the cytokine ~~gene~~ genes on the vector. The aforesaid ~~co-incorporation~~ construction can be achieved at least by ~~way of:~~ combining the tumor-associated ~~gene~~ genes and the cytokine ~~gene~~ genes into a fusion gene ~~to be~~ controlled by ~~the same~~ one promoter, for instance, fusing N neu gene and IL-2 gene behind a CMV promoter; or having two independent genes ~~that are~~ controlled ~~respectively~~ by two separate promoters respectively, for instance, inserting ~~respectively~~ N neu gene and IL-2 gene

respectively behind two separate promoters; or having two independent genes that are respectively controlled by a promoter and an IRES segment, for instance, inserting N neu gene behind CMV promoter and IL-2 gene behind IRES.

The aforesaid DNA vaccine ~~may be~~ is ~~carried~~ transformed ~~at least~~ by retroviral vector, adenoviral vector, adeno-associated viral vector, or liposome, or administered directly in the form of DNA. The aforesaid viral vectors offer higher transfection efficiency and better expression, but each of them has its limitation. For instance, retroviral vector can only transfect cells in division; adenoviral vector ~~tends to~~ leads to induce strong immune response; and adeno-associated viral vector has limited gene capacity. Non-viral vectors described above, such as liposome is very safe, but its transfection efficiency and expression are not as desirable as viral vectors. The proper vector for DNA vaccine of the present invention can be selected based on the ~~actual~~ practical needs. On the other way, ~~The~~ the DNA vaccine can put into practice by directly ~~may be injected~~ injecting DNA into muscle cells ~~cell directly in the form of DNA on account of the fact that~~, and muscle ~~cell~~ cells will automatically ~~ingest~~ uptake the DNA and express it, which is sufficient to elicit enhanced immune response despite of the relatively low expression level.

The DNA vaccine herein may be administered at least by way of subcutaneous injection, intramuscular injection, oral administration, spraying or gene gun injection.

BRIEF DESCRIPTION OF THE PREFERRED EMBODIMENTS

The preparation and efficacy of DNA vaccine in the present invention are further depicted with the illustration of embodiments.

EMBODIMENT 1 - Making a DNA vaccine by fusing N neu gene and mature IL-2 fragment

In this embodiment, DNA recombination technique is used to make a DNA vaccine containing N neu gene encoding the extracellular domain of neu protein and a mature IL-2 fragment. The preferred primer sequences for constructing the DNA vaccine of present invention, as used herein, includes the forward *Hind* -N'-neu primer (SEQ ID NO:1), the reverse N'-neu-*Not* primer (SEQ ID NO:2), the forward *Not* -IL2 primer (SEQ ID NO:3) and the reverse IL-2-*xba* primer (SEQ ID NO:4). Fig. 1 shows the preferred primer sequence for constructing a DNA vaccine ~~according to this invention that contains~~ containing the fusion of N neu gene and mature IL-2 fragment in the present invention. The steps include designing a proper restriction site and isolating the desired N neu gene and ~~mature~~ full length IL-2 fragment using polymerase chain reaction, and after restriction enzyme digestion, adding ligase to first constructing N neu gene on a mammalian expression vector pRC/CMV, and then using restriction enzyme and ligase to insert a ~~mature~~ full length IL-2 fragment downstream of N neu gene to form a DNA plasmid containing N neu-IL-2 fusion gene. As shown in Fig. 2, ~~said~~ the DNA plasmid is ~~inserted~~ transformed into *Escherichia coli* DH5α for mass reproduction and then extracted with Endofree Oiagen plasmid-Mega kits to complete the preparation of a DNA vaccine containing the fusion of N neu gene and mature IL-2 fragment.

EMBODIMENT 2 The tumor-suppressing effect of N neu-IL-2 fusion DNA vaccine

In this embodiment, ~~cohorts of~~ mice were injected with $1*10^6$/ml MBT-2 bladder cancer cells on the back to induce tumor growth. Ten days later, N neu-IL-2 fusion DNA vaccine prepared according to Embodiment 1 or normal saline (as control) was administered intramuscularly in the first time into the tumor site. The second and third administrations of vaccine took place on day 7 and day 14 after the first administration respectively (see Fig. 3 ~~for~~ referring to the flow ~~process~~ chart). The sizes of tumors measured at the ~~time of~~ first administration and 2-3 times each week afterwards are shown in Fig. 4. It is found that in comparison with normal saline, N neu-IL-2 DNA vaccine has ~~marked~~ remarkable tumor-suppressing effect.

EMBODIMENT 3 The effect of N neu-IL-2 fusion DNA vaccine on the survival rate of mice

In this embodiment, ~~cohorts of~~ mice were injected with $1*10^6$/ml MBT-2 cells on the back to induce tumor growth. Ten days later, tumor approximately $25mm^3$ in size grew from the injection site, and N neu-IL-2 fusion DNA vaccine prepared according to Embodiment 1 was administered intramuscularly in the first time into the tumor; some mice received normal saline or DNA vaccine containing only N neu, but not IL-2 as control groups. The second and third administrations of vaccine took place on day 7 and day 14 after the first administration respectively (see Fig. 3 for flow process). As shown in Fig. 5, all mice (37) administered with normal saline died in 56 days after being inoculated with MBT-2 cells (line a), while 8 of the 37 mice that received N neu DNA vaccine survived (survival rate of 22% as shown in line d), and 18 of the 37 mice that received N neu-IL-2 fusion DNA vaccine survived (survival rate of 49% as shown in line e). If the observation time was extended another three weeks, that is, 90 days after the inoculation of MBT-2 cells, only 4 out of 37 mice that were administered with N neu DNA vaccine survived (survival rate of 11%, as shown in line d), while 12 out of 37 mice that received N neu-IL-2 survived (survival rate of 32% as shown in line e). The results suggest that N neu-IL-2 fusion DNA vaccine is more

effective than N neu DNA vaccine in slowing down the growth of tumor, and more effectively prolonging the life of mice in the long run.

EMBODIMENT 4 Comparing the effect of N neu-IL-2 fusion DNA vaccine and the combination of N neu vaccine and IL-2 vaccine given separately on the survival rate of mice

To further demonstrate the progressive nature of the prevent invention, this embodiment compares the effect of N neu-IL-2 fusion DNA vaccine and the combination of N neu vaccine and IL-2 vaccine given separately on tumor suppression. The method of tumor cell injection and the time for administering DNA vaccines in this experiment are shown in Fig. 3. The results, as illustrated in line c and line e, show that 90 days after the mice were injected with MBT-2 cells, 12 out of 37 mice administered with N neu-IL-2 fusion DNA vaccine survived (survival rate of 32% as shown in line e), while only 1 out of 16 mice administered with combination of N neu vaccine and IL-2 vaccine given separately lived (survival rate of 6% as shown in line c), further indicating the superior effect of N neu-IL-2 fusion DNA vaccine.

The DNA vaccine of the present invention has been disclosed in the embodiments. However the embodiments should not be construed as a limitation on the spirit and scope of the appended claims .Those skilled in the art can easily understand that all kinds of alterations and changes can be made within the spirit and scope of the appended claims.

The DNA vaccine of the present invention containing tumor-associated gene and cytokine gene offers several advantages: (1) It can induce cellular and humoral immune responses which last for a long time; (2) the expressed antigen has a structure that approximates that expressed in human body during natural infection, thereby producing better immunization effect; (3) it can cross recognize different parts of the antigen, which helps overcome the problem of vaccine escape mutant; (4) combined immunization may be carried out by inserting combination of different antigen genes in the

plasmid; (5) it has a variety of administration routes, including subcutaneous, intramuscular, oral, spray or gene gun; and (6) its preparation process is simple and cost-effective. It is also easy to mass produce, transport and preserve. It is an improvement over known cancer-treating vaccines and demonstrates better efficacy.

ABSTRACT

The present invention discloses a DNA vaccine and ~~its~~ the method of preparation preparing the same, which employs DNA recombination technique to co-incorporate a fragment of tumor-associated gene and a fragment of cytokine gene into a vector. The administration of such DNA vaccine ~~may~~ can enhance the immune response of the host through the co-expression of tumor-associated ~~antigen~~ protein and cytokine, and thereby achieve the therapeutic effect of inhibiting or retarding tumor growth.